

Determination of Chlorpyrifos – A New Approach

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Abstract

Spectrophotometric and gas chromatographic methods were developed for the determination of chlorpyrifos. Alkaline hydrolysis of chlorpyrifos to 1,2,4-trichloropyridine was the basis followed by coupling with diazotized aniline in presence of nitric acid. The absorption maxima of blue color formed at 490nm. Beer's law was obeyed in the range of 8-48 µg/ml. The standard deviation was found to be ± 0.005 . The method was free from other pesticide interferences and applied to various fruits and vegetables procured at various agricultural fields at sabbavaram area, Visakhapatnam, India and found satisfactory. Determination of pesticide residue in the various field water, vegetables collected are eggplant, cauliflower, potato, spinach, and fruits such as pine apple, grapes etc were analysed by using Gas chromatography with electron capture detector (GC-ECD). Residues were extracted with hexane, dichloromethane and ethyl acetate (1:1:1 V/V). Clean up procedure was carried out by applying solid phase extraction column. The recoveries of chlorpyrifos pesticide in fruits and vegetables selected were in the range of 80 to 98% fortified at 0.5mg/Kg. Some grape samples showed above the limit of quantification.

Keywords

Chlorpyrifos; Diazotised Aniline; Spectrophotometry/ GC-ECD; Fruits; Vegetables

Introduction

Chlorpyrifos is a crystalline organophosphate insecticide. The IUPAC name of chlorpyrifos is O, O-diethyl O-3,5,6-trichloro-2-pyridyl phosphorothioate and with molecular formula $C_9H_{11}Cl_3NO_3PS$. Chlorpyrifos is moderately toxic and chronic exposure has been linked to neurological effects, developmental disorders, and autoimmune disorders. Chlorpyrifos is manufactured by reacting 3,5,6-trichloro-2-pyridinol with diethylthiophosphoryl chloride [Muller et.al(2000)] and then registered only for agricultural use, where it is "one of the most widely used organophosphate insecticides", according to the United States Environmental Protection Agency (EPA). The crops with the most intense chlorpyrifos use are cotton, corn,

almonds, and fruit trees including oranges and apples produced via a multistep synthesis from 3-methylpyridine. Chlorpyrifos is an organophosphate, with potential for both acute toxicity at larger amounts and neurological effects in fetuses and children even at very small amounts. Recent research indicated that children exposed to chlorpyrifos while in the womb have an increased risk of delays in mental and motor development at age 3 and an increased occurrence of pervasive developmental disorders such as ADHD [Rauh et.al(2006)]. An earlier study demonstrated a correlation between prenatal chlorpyrifos exposure and lower weight and smaller head circumference at birth [Whyatt et.al.(2004)]. A study of the effects of chlorpyrifos on humans exposed over time showed that people exposed to high levels have autoimmune antibodies that are common in people with autoimmune disorders. There is a strong correlation to chronic illness associated with autoimmune disorders after exposure to chlorpyrifos [Thrasher et.al (2002)]. The measuring of urinary metabolite TCP of chlorpyrifos via biological monitoring would be useful, allowing comprehensive evaluation of the exposure to chlorpyrifos in indoor air [Hong dai. et.al (2003)]. Chlorpyrifos is used for termite control in construction, forestry and field crops. Numerous instrumental methods have been described for the detection and determination of chlorpyrifos generally analysed by spectrophotometry [Elosa d et.al (2001), Janghel et.al(2007)], thin layer chromatography (TLC) [Patil v.b.et.al(1994)], and GC-MS [Thanh et.al (2008)], liquid chromatography, mass spectrometry [Blasco(2004)], and Gas chromatography [A.Bouaid (2010), venugopal(2012)]. The purpose of this study was to develop a spectrophotometric method and an analysis scheme for determination of chlorpyrifos in cauliflower, potato, spinach, and fruits such as pink apple, grapes etc by GC-ECD. In this paper the author extracted chlorpyrifos pesticide in fruits and vegetables using hexane, hexane-diethyl ether, hexane and dichloromethane as solvents, clean up on solid phase extraction (SPE) and determined on Gas Chromatography with electron

capture detector(GC-ECD).

Experimental

Instrument

A Jasco (Model Uvidec-610) UV-VIS Spectrophotometer with 1cm matched quartz cuvettes and Agilent 6820 gas chromatograph were used for all measurements. Nitrogen (99.9%) carrier gas and Intercap column (30m length, 0.25mm dia 0.25 mic.DF) were used.

Reagents and Chemicals

Standard Solution of chlorpyrifos: 200ppm solution of chlorpyrifos in methanol was prepared by dissolving 0.02 gm of chlorpyrifos standard in required volume of methanol and 100ml volumetric flask with double distilled water.

Diazotization of Aniline: 2gm of aniline was dissolved in a mixture of 5.5ml of concentrated hydrochloric acid and 5.5ml of water contained in a 100ml round bottomed flask. The solution was immersed in ice so that the temperature of the stirred solution fallen below 5°C. Dissolve 1.6g of sodium nitrite was dissolved in 7.5ml of water and chilled the solution by immersion in the ice bath. To the cold aniline hydrochloride solution small volume of cold sodium nitrite solution was added. Benzene diazonium chloride solution is formed.

2M aqueous solution of sodium hydroxide was prepared.

All other chemicals used were of Analytical reagent grade from Merck Company.

Sampling

Several samples of water, fruits and vegetables were collected from Agricultural fields in Sabbavaram, Visakhapatnam district. Samples of one kilogram grapes, pine apple (fruits) and carrot, cabbage, (vegetables) were procured and kept in refrigerator till analysis.

Procedure

Into a series of 25ml volumetric flask containing aliquots of standard chlorpyrifos solution (200ppm), 1.0ml of 2M sodium hydroxide was added. The solution was kept for half an hour for complete hydrolysis and 0.5ml of diazotized aniline was added. The solution was kept aside for ten minutes for complete color development. Now the volume was made up to the mark with distilled water. The solution was stable and the wine red solution was measured at 490 nm against a reagent blank.

Extraction and clean up

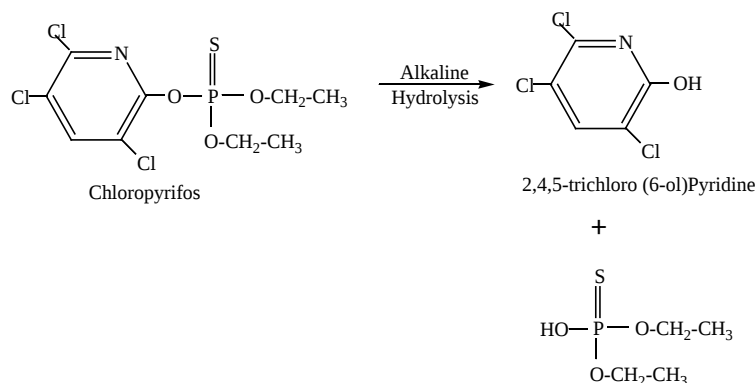
The samples collected from Agricultural fields of Sabbavaram and Chintalaagraharam areas of Visakhapatnam district, Andhra Pradesh, India were procured and kept in refrigerator till analysis. Each vegetable was chopped into small pieces. A representative sample (50gm) was taken with 5-10gm anhydrous sodium sulphate in blending machine to make fine paste. The sample was extracted with 100ml of hexane, ethyl acetate or dichloromethane on mechanical shaker for one hour, and then filtered, concentrated up to 5ml on rotary evaporator and finally injected into GC-ECD. The clean-up of chlorpyrifos was performed out by using column chromatography packed with Florisil. Recoveries of pesticides were determined by comparison of the ratio of the peak area of the analyte against the peak area of the internal standard from the spiked samples with that of the standard calibration solutions.

Results and Discussion

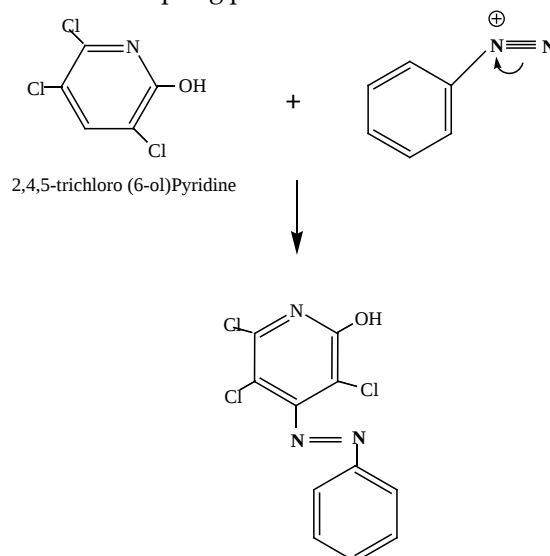
Proposed scheme;

Step 1:

i) Alkaline hydrolysis of chlorpyrifos



Step 2: Diazo coupling product



To establish the optimum wavelength of maximum absorption ($\lambda_{\max}$) of the colored species formed in the above method, specified amount of chlorpyrifos was taken and the color was developed. The absorption spectra were scanned on a spectrophotometer in the visible region against reagent blank. The UV-Visible absorption spectrum for the solution containing intense red color showed a characteristic, intense peak at 490nm in aqueous solution and molar extinction coefficient was about 0.7888×10^4 [L]/[mol].[cm]. The quantitative results were obtained at $44 \mu\text{g/ml}$. The recommended method was applied to different vegetable and fruits samples. Very low values were obtained and the color formed was stable for few minutes.

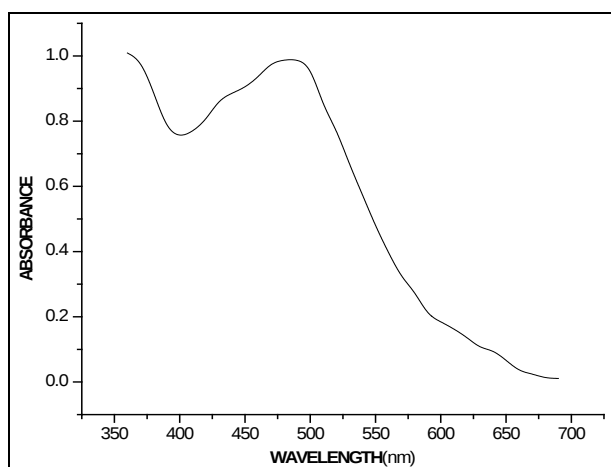


FIGURE 1 ABSORPTION SPECTRUM OF CHLORPYRIFOS-DIAZOTIZED ANILINE SYSTEM

Optical Characteristics

The Beer's law at appropriate lengths of a set of solutions containing variable amounts of chlorpyrifos and specified amount of diazotized aniline was recorded against the corresponding reagent blank. The Beer's law plot recorded graphically in figure 2. Beer's law limits, Molar absorptivity, Sandell sensitivity for chlorpyrifos are calculated in table 1. To get correlation coefficient value, least squares regression analysis was carried out. The precision of the proposed methods was ascertained from the absorbance values obtained by actual determination of six replicates of a fixed amount of chlorpyrifos in total solution. The percent relative standard deviation was calculated for the proposed method given in table 1.

The proposed method attempted to resolve the troubles involved in analysis of pesticides residues. Injection of ten microlitres increased sensitivity enough to improve the detection limit for the method for max-

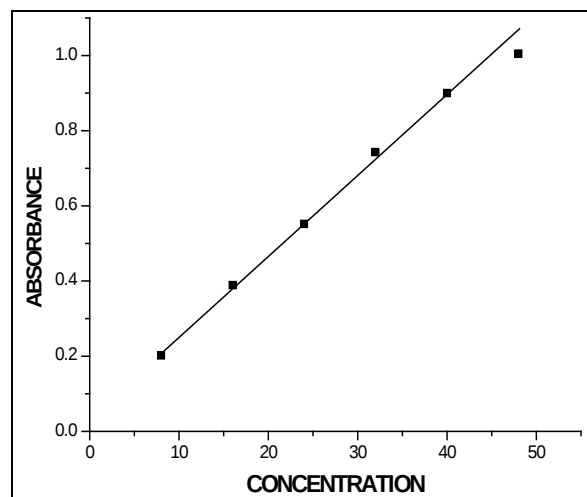


FIGURE 2 BEER'S LAW PLOT OF CHLORPYRIFOS-DIAZOTIZED ANILINE SYSTEM

TABLE 1 OPTICAL AND REGRESSION CHARACTERISTICS FOR CHLORPYRIFOS

Parameters	Chlorpyrifos-Diazotised aniline
$\lambda_{\max}(\text{nm})$	490
Beer's law limits ($\mu\text{g/ml}$)	8-48
Molar absorptivity ($1 \text{ mol}^{-1} \text{ cm}^{-1}$)	0.7888×10^4
Sandell's sensitivity ($\mu\text{g cm}^{-2}/0.001 \text{ absorbance unit}$)	0.0444
Correlation coefficient (r)	0.9967
Relative standard deviation (%)**	0.06318

** Six replicate samples

imum residue levels. Injecting vegetable extracts was very complicated and must be carefully considered. The pesticide residues present in the fruits and vegetables samples were identified and quantified with reference to standard pesticides. The calculation of the amount of the pesticides present was carried out by comparing the peak areas for unknown samples with the corresponding peaks for standards, according to established procedures in the process of development of GC methods for high resolution of chromatographic peaks in order to reach lower limits of detection. Selective and sensitive detectors, as in ECD, provided good responses even to very low concentrations.

Method Optimization

Preliminary experiments were performed by direct injection of pesticides for GC-ECD conditions optimization and the temperature program developed was capable of a good separation of the investigated analytes. Twelve samples of water, fruits and vegetables were collected from Agricultural fields in Sabbavaram, Visakhapatnam district for the determination of chlorpyrifos tabulated in the below table 2.

The present method was tailor-made in view of the previous information about the most prevalent pesticides in the area. From the results it can be seen that chlorpyrifos was detected in the range of 0.13-0.84 mg/kg. The recoveries for chlorpyrifos in various samples ranged from 80-98% with coefficient of variance 1.3-7.6%. The various chromatograms for the determination of chlorpyrifos were given in figures3-5.

Conclusion

GC-ECD has great potential for determining pesticides in food extracts at low levels. In general, chlorpyrifos residues in food are well below the tolerance levels established for various food types by the FAO/WHO.

TABLE 2 CHLORPYRIFOS PESTICIDE RESIDUE PRESENT IN PPM IN VARIOUS WATER, FRUITS AND VEGETABLES SAMPLES

S.No	Name of the item		Pesticide residue (mg/Kg)
1.		Water	0.31,0.22
		Banana field	0.32,0.36
		Rice field	0.75,0.76
2.	Fruits	Pine-apple	0.70,0.75
		Grapes	0.83,0.76
3.	Vegetables	Carrot	0.21,0.08
		Cabbage	0.26,0.23
		Spinach	0.47,0.34
		Tomato	0.84,0.76
		Okra	0.39,0.35
		Brinjol	0.62,0.66

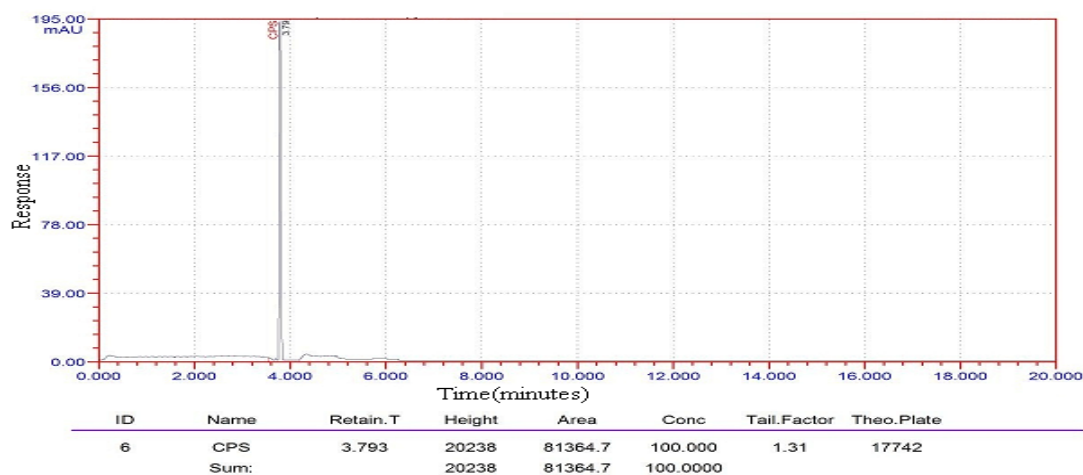


FIGURE 3 GC CHROMATOGRAM OF CARROT EXTRACT AFTER CLEAN UP

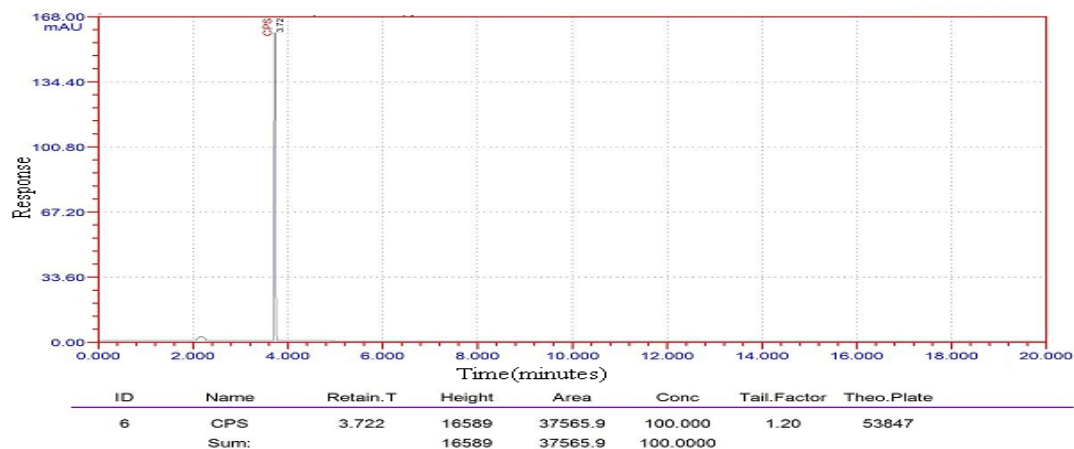


FIGURE 4 GC CHROMATOGRAM OF OKRA EXTRACT AFTER CLEAN UP

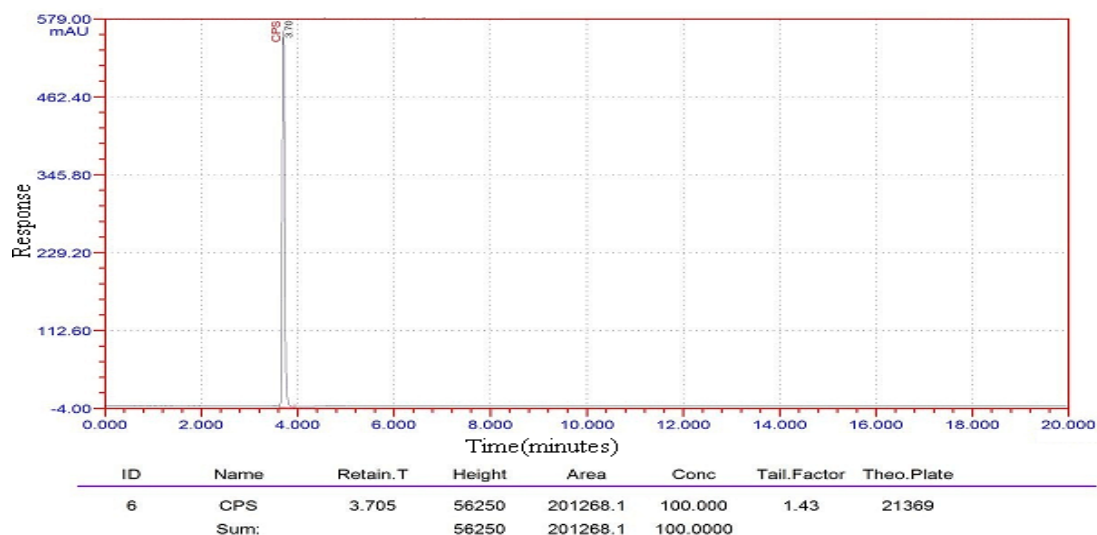


FIGURE 5 GC CHROMATOGRAM OF CABBAGE EXTRACT AFTER CLEAN UP

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